BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problems Mailbox.

VOLUME ONE

CANCER MEDICINE

Fourth Edition

James F. Holland
Distinguished Professor of Neoplastic Diseases
Director Emeritus, Derald H. Ruttenberg Cancer Center
Mount Sinai Medical Center
New York, New York

Robert C. Bast, Jr.
Internist and Professor of Medicine
Harry Carothers Wiess Chair for Cancer Research
Head, Division of Medicine
University of Texas M. D. Anderson Cancer Center
Houston, Texas

Donald L. Morton
Medical Director and Surgeon-in-Chief
John Wayne Cancer Institute at St. John's Hospital
Santa Monica, California
Pr fessor and Chief, Emeritus, Surgery/Oncology
UCLA School of Medicine
Los Angeles, California

Emil Frei III
Director and Physician-in-Chief, Emeritus
Dana-Farber Cancer Institute
Richard and Susan Smith Distinguished Professor of Medicine
Harvard Medical School
Boston, Massachusetts

Donald W. Kufe
Deputy Director
Dana-Farber Cancer Center
Chief, Division of Cancer Pharmacology
Dana-Farber Cancer Institute
Professor of Medicine
Harvard Medical School
Boston, Massachusetts

Ralph R. Weichselbaum
Harold H. Hines, Jr., Professor and Chairman
Department of Radiation and Cellular Oncology
University of Chicago Hospital
Director, Chicago Tumor Institute
University of Chicago
Chicago, Illinois

Williams & Wilkins
A WAVERLY COMPANY

BALTIMORE • PHILADELPHIA • LONDON • PARIS • BANGKOK
BUENOS AIRES • HONG KONG • MUNICH • SYDNEY • TOKYO • WROCLAW

Editor: Jonathan W. Pine, Jr.
Managing Editor: Molly Mullen
Production Coordinator: Carol Eckhart
Copy Editor: Hockett Edit rial Service
Designer: Norman Och
Illustration Planner: Raymond Lowman
Cover Designer: Dan Pfisterer
Typesetter: Maryland Composition Company, Inc.
Printer and Binder: R. R. Donnelley & Sons

351 West Camden Street
Baltimore, Maryland 21201-2436 USA

Rose Tree Corporate Center
1400 North Providence Road
Building II, Suite 5025
Media, Pennsylvania 19063-2043 USA

Accurate indications, adverse reactions and dosage schedules for drugs are provided in this book, but it is possible that they may change. The reader is urged to review the package information data of the manufacturers of the medications mentioned.

Printed in the United States of America

First Edition, 1973
Second Edition, 1982
Third Edition, 1993

Library of Congress Cataloging-in-Publication Data may be obtained from the Publisher.

ISBN 0-683-04095-2

The publishers have made every effort to trace the copyright holders for borrowed material. If they have inadvertently overlooked any, they will be pleased to make the necessary arrangements at the first opportunity.

To purchase additional copies of this book, call our customer service department at (800) 638-0672 or fax orders to (800) 447-8438. For other book services, including chapter reprints and large quantity sales, ask for the Special Sales department.

Canadian customers should call (800) 268-4178, or fax (905) 470-6780. For all other calls originating outside the United States, please call (410) 528-4223 or fax us at (410) 528-8550.

Visit Williams & Wilkins on the Internet: http://www.wwilkins.com or contact our customer service department at custserv@wwilkins.com. Williams & Wilkins customer service representatives are available from 8:30 am to 6:00 pm, EST, Monday through Friday, for telephone access.

97 98 99 00 01
1 2 3 4 5 6 7 8 9 10

CHAPTER 56

Animal Models in Drug Development

SAMIR N. KHLEIF AND GREGORY A. CURT

Introduction

The process of cancer drug discovery may begin with either empiric screening or rational drug design. In either case, the necessary steps in drug development that follow the identification of an interesting lead require appropriate animal model systems. Just as screening systems and rational drug design have benefited from recent advances in cell culture technique and molecular biology, so too has the role of animal model systems in drug development. Beyond simply predicting dose-limiting toxicity, drug metabolism or tissue and compartment distribution, animal models are increasingly being used to guide dose escalation in Phase I trials and provide tumor microenvironments that mimic the clinical situation.

The processes of cancer drug discovery and drug development have evolved, and will continue to change, since the first successful use of drugs to treat systemic cancer more than 50 years ago. Basic research in cancer biology has provided new targets for cancer drug development and brought older targets into sharper focus. Of the properties that make a cell malignant (uncontrolled growth, metastasis, dedifferentiation, genetic plasticity, and drug resistance), only uncontrolled growth has been exploited as a target for cancer drug development. Agents that have the potential to interfere with the metastatic cascade, interrupt autocrine and paracrine growth loops, differentiate tumors, or reverse drug resistance are now in preclinical development and early clinical trial. Appropriate and evolving animal model systems will be needed to discover the next generation of cancer drugs and bring them to clinical study. This chapter will discuss the history and future of cancer drug discovery and drug development with special emphasis on the role of animal models in the process.

THE ROLE OF ANIMAL MODELS

In Drug Discovery

Drug Screening. The idea that systemic drugs could treat, and possibly cure, systemic cancer is relatively new in medicine. In the mid 1940s, Gilman's treatment of lymphomas with alkylating agents at Yale and Farber's induction of short remissions in leukemia with antifolates at Harvard led the National Cancer Institute (NCI) to begin a major effort in cancer drug discovery and development. Stated in its simplest terms, the purpose of the initial NCI screen was to select and prioritize drugs for clinical trial (4–6, 17–23, 33, 38, 8, 113, 168, 169).

In 1955, murine leukemia models P388 and L1210 were selected as the initial system in which potential agents would need to demonstrate activity before further development. The reason for this selection was simple. Murine leukemia and lymphoma models were relatively inexpensive and allowed for a relatively high throughput of compounds. Indeed, from the inception of the mouse screen until its first modifications in the mid 1970s, more than 400,000 compounds passed through this screen.

At first, this mouse screening system was empiric. Over time, however, this empiricism became more enlightened with the development of the NCI Drug Information System. This computer-based inventory maintains the structure of each compound screened and its activity in murine model systems. This system has been used to limit the screening of analogues while turning greater attention to novel structures. Importantly, the Drug Information System also maintains discrete databases on compounds provided to NCI on a proprietary basis by pharmaceutical companies, allowing open access of the screen to industry.

From the beginning, however, it was obvious that this system had serious limitations. While most of the active drugs currently used in the treatment of leukemia and lymphoma were initially screened in the L1210 system, screening against rapidly growing leukemic cells could bias selection toward compounds that are preferentially active against rapidly growing tumors with essentially a 100% growth fraction. In fact, it was found that plateau phase cultures were less sensitive to cycle-specific agents than log phase culture, while some classes of clinically useful drugs, such as the alkylators, were active in plateau phase cell lines. The development of drugs active against the solid tumors of adulthood would presumably require a different approach.

The availability of new rodent models enabled the NCI to take further steps toward rational drug screening in 1975 (63). Instead of a single hurdle of activity in murine leukemia, compounds active in this system were subsequently tested against a panel that included transplantable murine tumor models designed to resemble common human solid tumors (including melanoma and lung, colon, and breast cancer) both in histology and cell kinetics. In a step that would presage later changes in the NCI screen, the availability of athymic (nude) mice also allowed the screening of drugs against transplantable human tumors as well (138, 162). Initially, these human tumor xenografts included lung, colon, and breast cancer (52, 97, 121).

Overall, these changes took the NCI screen from a com-

Table 56.1. Origin of DCT Prescreen and Tumor Panel Mod ls

Site tumor	Host of origin	Tumor of origin	Historical description	Site
		Prescreen		
P388 leukemia	DBA/2 mouse	Chemically induced with 3-methyl-cholanthrene	Lymphocytic leukemia	IP
L1210 leukemia	DBA/2 mouse	Chemically induced with 3-methyl-cholanthrene in ethyl ether	Lymphoid leukemia	IP
		Tumor Panel		
Mouse tumors				
B16 melanoma	C57BL/6 mouse	Spontaneous at base of ear	Melanoma	IP
CD8F mammary carcinoma	CD8F female mice	Spontaneous	Mammary adenocarcinoma	SC
Colon 38	C57BL/6 mouse	Induced by 1,2-dimethylhydrazine	Colon carcinoma	SC
Lewis lung carcinoma	C57BL/6 mouse	Spontaneous in the lung	Carcinoma	IV
Human tumor xenografts				
CX-1 colon	Isolated in tissue culture, subsequently maintained in nude mice	Human colon. Untreated primary tumor from 44-year old caucasian female	Adenocarcinoma of the colon	src
LX-1 lung	Isolated and maintained in nude mice	Metastatic lesion from arm of 48-year-old male with oat cell lung carcinoma treated with *Corynebacterium Parvum*, cyclophosphamide (Cytoxan), and radiation.	Carcinoma	src
MX-1 mammary	Isolated and maintained in nude mice	Human breast. Primary tumor from 29-year-old female with no previous chemotherapy. CL-1 line	Carcinoma	src

src, subrenal capsule; IP, intraperitoneal; IV, intravenous; SC, subcutaneous.

pound-oriented toward a more tumor-specific approach. However, the high cost of the transplantable mouse and human xenograft systems (approximately $5,000 per compound) was unsuitable for high capacity screening. Instead, the NCI designed a two-stage system in which the murine leukemia model was maintained as a stage I "prescreen."

Compounds entering the system were first tested against a highly drug-sensitive mouse leukemia. Agents shown active against P388 or L1210 were then tested in a stage II screen against the solid tumor panel. Table 56.1 illustrates the animal tumor panel used in the screen. It includes both the transplantable murine tumor models and the human tumor xenografts. In turn, those agents with the broadest spectrum of activity against solid tumors received priority for phase I clinical trial (Fig. 56.1).

The rationale for this approach was simple. Earlier experience in the NCI murine leukemia-based screen had shown that L1210 and P388 were the most sensitive models for drugs that were subsequently shown to have clinical activity (138). In particular, P388 was more sensitive to compounds of the natural product class than L1210 (64, 148). Thus, most inactive compounds could be screened-out by an inexpensive, high capacity, highly sensitive, less specific prescreen before the presumably more rigorous development in the low capacity, less sensitive, more specific tumor panel. When in full operation, P388 screened some 15,000 compounds each year, of which 500 to 1,000 were advanced into the stage II (solid tumor) phase of testing.

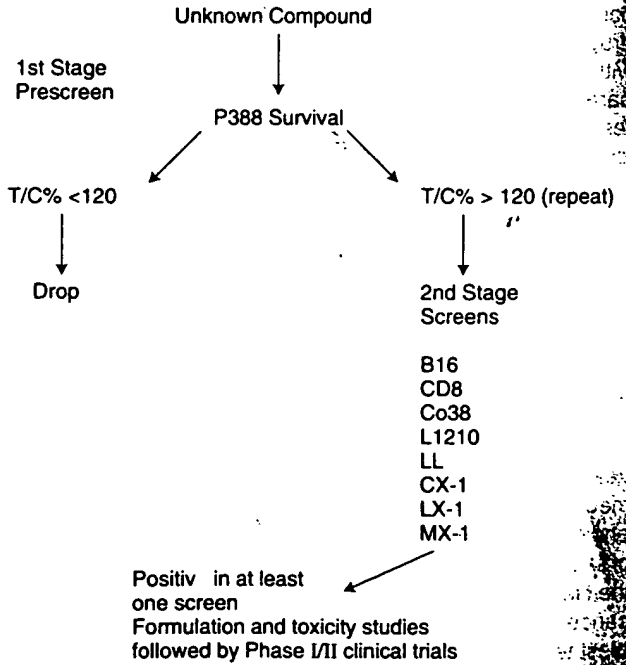


Figure 56.1. National Cancer Institute Drug Screening Strategy, 1975–1985.

While the approach appeared a reasonable compromise considering costs and logistics, the limitations remain obvious. The refined screen remained, at its heart, a compound-oriented strategy using a highly sensitive, rapidly dividing leukemia model for initial intake. However, the bias against selection of drugs specifically active in solid tumors remained. For example, fewer than 2% of all agents active against P388 showed significant effects in Lewis lung or colon (38) adenocarcinomas (91, 148).

Most disturbing was the failure of the disease-specific phase II component of the screen to predict for disease-specific clinical activity. Retrospective analysis of solid tumor activity in phase II trials was not predicted by parallel preclinical solid tumor activity. As will be discussed later, if a given drug demonstrated significant preclinical activity in xenograft models of human breast, colon, or lung cancer, this did not predict for clinical activity in patients with these diseases. In addition, the screen identified few active new leads (11, 30, 51, 163). Accepting the limitations of animal models of this type in cancer drug screening and recognizing the need for a preclinical screening system with greater predictive power, the NCI began to focus on a truly disease-oriented approach to drug discovery in the mid 1980s. Ideally, such a screen would be able to detect broadly active or disease-specific drugs.

Because of the high cost and a continued need for a high volume screen, animal models were determined to be impractical. Instead, the development, characterization, and maintenance of an entirely in vitro human tumor cell line screen was initiated (7, 8, 37, 103, 107, 141, 155). In 1985, the NCI screen evolved into its most recent configuration, an in vitro (stage I) screen followed by the more refined in vivo (stage II) screen (Fig. 56.2).

In stage I, agents are tested against a panel of approximately 60 cell lines representing the most common solid tumors of adulthood including lung, breast, colon, renal, and ovarian cancer (21, 23). Drug-resistant tumors are specifically included in the screen. These include the human breast carcinoma selected for multiple drug resistance (mdr) and P388 murine leukemia resistant to natural products, both of which potentially provide additional identification of new agents with particular activity against potentially resistant tumors (31, 71). In stage II, the most sensitive human tumors are tested against the same drug in nude mice. Activity in the nude mouse-human xenograft system in itself is sufficient for further preclinical development including toxicology and formulation, steps that are often the most costly process of drug development.

In summary, the current NCI approach to cancer drug discovery has evolved from a highly empiric compound oriented animal-based screen to a human in vitro panel. An advantage to this approach in addition to its disease orientation and adaptability to high volume screening is its flexibility with respect to natural product extracts. While animal models require relatively large quantities of relatively pure compounds for screening, the in vitro panel can actually be used to purify active compounds from small quantities of natural product extracts.

However, animal models will continue to play a critical role in cancer drug development. Preclinical activity of an antitumor agent in a relevant in vivo system is a sine qua non for clinical testing. As development of anticancer agents turns progressively toward agents that modify biological responses, differentiate tumors, and inhibit metastasis and invasion, animal models will become more important in the future. Immunostimulants and inhibitors of metastasis can only be studied preclinically in the appropriate animal model system.

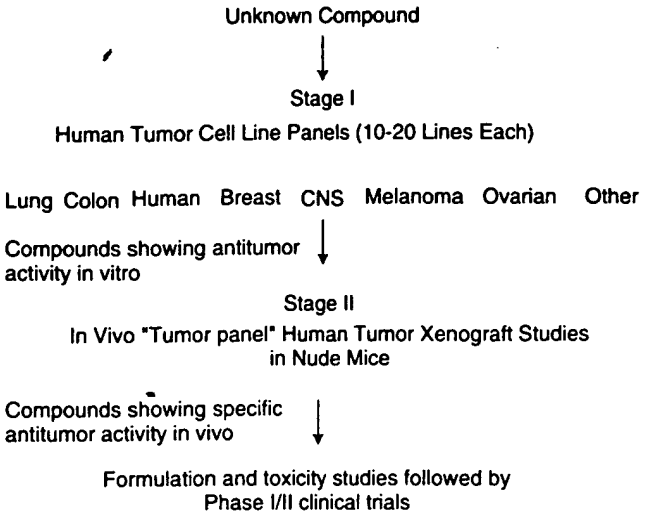


Figure 56.2. National Cancer Institute Drug Screening Strategy 1985–present.

ANIMAL TUMOR MODELS

The selection of the appropriate experimental model is critical to cancer drug discovery and development. The value of the model depends on its validity, selectivity, predictability, and reproducibility (33, 34, 168). In cancer drug development, the animal model is selected to demonstrate the cytotoxic effect of the drug or biological agent on the tumor passage in that model system.

There is no perfect tumor model for any human cancer. Nevertheless, in selecting the best model system, consideration should be given to the genetic stability and heterogeneity of the transplanted cell line, its immunogenicity within the host animal, and the appropriate biologic endpoint (local growth, metastasis, survival). For example, the KHT sarcoma is a tumor with high metastatic potential, making it a very suitable model for the evaluation of a combined modality treatment (164) or inhibitor of metastasis.

In general, animal tumor models can be divided into either spontaneous or artificially transplanted systems. Solid tumors are usually transplanted by the inoculation of cell suspensions by the subcutaneous (SC), intradermal (ID), intramuscular (IM), intraperitoneal (IP), or intravenous (IV) routes. Leukemia models are transplanted only by the SC, IV, or IP routes.

The spontaneous tumor models that are either idiopathic or arise following carcinogenic (28, 29) or viral exposure mimic the clinical situation most closely. Spontaneous tumors are usually measurable only late in their course. Their metastatic pattern is not uniform, and their response to therapy is generally poor. They also resemble human cancers in kinetics and antigenicity.

However, there are significant obstacles to the use of such model systems. For example, a relatively small percentage of animals may develop disease following exposure to carcinogen or virus, and the tumors may have a variable natural course. In addition, the inability to establish accurate staging makes these models quantitatively unsuitable for assessing therapeutic response to an agent given in a uniform fashion. Generally speaking, spontaneous tumor models have their greatest role in studying the biology of carcinogenesis. In the future, they may also be important in the development of chemopreventive or chemosuppressive drugs.

The models with the widest use in experimental therapeutics are the transplanted animal tumor models and the human tumor xenografts. These will be discussed in some detail below.

Transplantable Animal Tumor Models

Early passages of transplanted tumors resemble spontaneous cancer most closely. These early passages show significant heterogeneity in cell kinetics and histology (101, 151). Despite these limitations, such models have been used in drug screening. Because established transplantable tumor models are well characterized and reproducible, they have traditionally been the foundation of cancer drug development (108, 131, 132, 144). How good are they in predicting clinical activity?

Multiple studies have been undertaken to assess the ability of preclinical animal activity to predict antitumor response in man (11, 14, 89). Marsoni and co-workers evaluated the activity of all cytotoxic drugs introduced into phase II clinical trial by the NCI between 1970 and 1985 (99). Of the 75 drugs entered into clinical trial during this period, 24 showed some evidence for clinical activity. One interpretation of these data is that the screen is highly predictive for clinical activity. Approximately 30% of drugs taken to clinical trial showed some evidence of activity. However, 74% of the drugs were active against lymphoma and 35% were active against leukemia. Only minimal activity was observed against solid tumors including those represented in the phase II portion of the screen. Indeed, analysis showed a poor correlation between preclinical in vivo and clinical activity in the same tumors. One must conclude that either animal model systems using transplantable tumors do not predict for clinical activity or that the P388 prescreen effectively selected against compounds specifically active in human solid tumors. The new in vitro human cell line screen will be important in answering these questions, since the initial identification of activity is in a human solid tumor rather than a murine leukemia-lymphoma model system.

A range of methods can be used to evaluate drug effect on tumors in animal models. Tumor size and tumor weight or volume changes are simple and easily reproducible parameters. Morphologic changes and alterations in tumor immunogenicity or invasiveness are other markers of response (62).

In addition, many specific assays have been developed for the measurement of treatment effects on tumors. This section will discuss some assays that can be used to judge tumor response.

Excisional Clonogenic Assay. This assay has been used widely as a method to assess what fraction of cells in a tumor population retain proliferative capability after being exposed to a chemotherapeutic agent. This assay is based on the assumption that the proliferative or clonogenic potential of tumor cells reflects the in vivo tumorigenicity of the tumor stem cell (149, 152). Thus, colony number is assumed to be proportionate to the number of viable cells.

The assay itself is straightforward. Tumor-bearing animals are tested with the drug under evaluation. At 24 hours, the tumors are excised from treated and untreated animals. A cell suspension is prepared from every tumor. The proliferative capacity of the cells in each suspension is evaluated by either in vivo inoculation intravenously into test animals of a selected cell suspension dilution (76, 153), or by plating the cells in liquid or agar medium (26, 75, 133). If an animal model is used, colony count is then performed in specific tissues at necroscopy. The lung, liver, and spleen are commonly used for this purpose. If the cells were plated in agar, a colony count is performed in the dish. Colony-forming efficiency (CE) of the inoculated cells is calculated to assess the efficacy of treatment in terms of cell survival.

$$CE = \frac{\text{number of tumor colonies counted}}{\text{number of tumor cells plated}}$$

The ratio of the CE treated to the CE control is called surviving fraction (SF).

$$SF = \frac{CE\ \text{treated}}{CE\ \text{control}}$$

SF is the best parameter for expressing cell survival results from the excisional biopsy (92, 154).

This assay has the advantage of placing the treated and untreated tumors in identical environments. It is also able to select a resistant population of cells within the tumor at a low drug dose. In addition, excising the tumor 24 hours after exposing the animal to the cytotoxic agent allows giving doses up to the transplant range, which has important implications for the selection of agents for bone marrow transplantation.

TD50 (Endpoint Dilution Assay). TD50 (74, 77, 93) is the tumor cell inoculum that produces tumor growth in 50% of inoculated animals or sites. It is a measurement of the number of cells required to produce tumors from inocula in vivo. The assay is based on the same principles as that of colony formation. A cell suspension is prepared from both treated and untreated animals, with ranges of dilutions for each tumor depending on the expected value of TD50. The suspension is inoculated into groups of test animals subcutaneously, intramuscularly or intradermally for solid tumors, and intraperitoneally or intravenously for leukemias. The percentage of tumor take versus cell number inoculated for each treatment is determined and compared to control animals to determine TD50 (49).

Tumor Growth Delay Assay. Cytotoxic treatment can slow tumor growth and delay disease progression. These effects are measured by the tumor growth delay assay (9, 10, 157). Tumor delay by definition is the time required for the treated tumor to reach a specific size minus the time for the untreated tumor to reach that certain size. This assay involves a very simple technique, little equipment, and can be

completed for many types of tumors before animals are lost to metastasis or disease progression. Unlike the survival time assay discussed later, this evaluation does not require death as an endpoint.

The correlation between the growth delay and the amount of cell kill varies with the growth rate of the tumor (10). Thus, when a treatment effect on tumors with different growth rates is assessed, a comparison of absolute growth delay between tumor models is misleading. Therefore, a specific growth delay (growth delay/doubling time of the tumor) reflects more accurately the differences in cell kill. Figure 56.3 illustrates the concept of specific growth delay.

Survival Time Assay. Another parameter that can be used to assess the effect of a drug on tumor in the animal model is the survival time. Survival time is an obvious endpoint, since it combines the sum total of interactions between tumor, drug, and host. Since drug toxicity and tumor growth both have independent effects on survival, a judgment can be made about therapeutic index. However, this approach cannot directly assess cell kill or time-dependent cytotoxicity.

The therapeutic efficacy can be assessed by determining the increase in survival as an effect of the escalating dose of the studied drug. As the dose of an active drug increases, the survival time increases because of increasing logarithmic tumor cell kill. Survival time reaches a maximum point as the toxic effect of the drug outweighs the therapeutic effect and survival times diminish (145). The maximum point of survival is called the optimal point (OP) or the maximum increase in life span (IL). The higher the OP the better the given intervention's therapeutic efficacy. This model also helps in assessing the safety of certain drugs by measuring the therapeutic ratio (TR), that is, the ratio between the optimal dose and that dose that leads to a specific increase in survival time (e.g., IL 20, IL 40, and so on). Therefore, in comparing drugs with the same maximum survival (optimal point), the higher the therapeutic ratio, the safer the drug (145).

A common use of survival to assess drug efficacy or increase in life span is the *T/C* percent ratio. This is defined as the ratio of the survival time of treated animals to the survival time of control, expressed as a percentage. This parameter has been used by the NCI for decision making, setting specific criteria of activity before further development is undertaken. A *T/C* of >120 in the solid tumor panel has been used as the benchmark for clinical development (Fig. 56.1) (15, 16, 100, 122).

Animal Tumor Xenografts

Before the availability of athymic or nude mice, human tumors were xenografted in mice immunocompromised by irradiation, thymectomy, or steroids (32, 158, 159). The first nude mice arose spontaneously in a closed, but not inbred, colony of albino mice in a virus laboratory in Ruchill Hospital, Glasgow, Scotland (134) and were described by Isaacson and Cattanach as lacking fur (84). The first xenograft in nude mice was performed by Rygaard and Povlsen in 1969 using a human colon adenocarcinoma (136).

Flanagan initially described the genetic component of immunodeficiency in this important model. He found that the mutant gene (*nu*, for nude) is present on chromosome 11, as

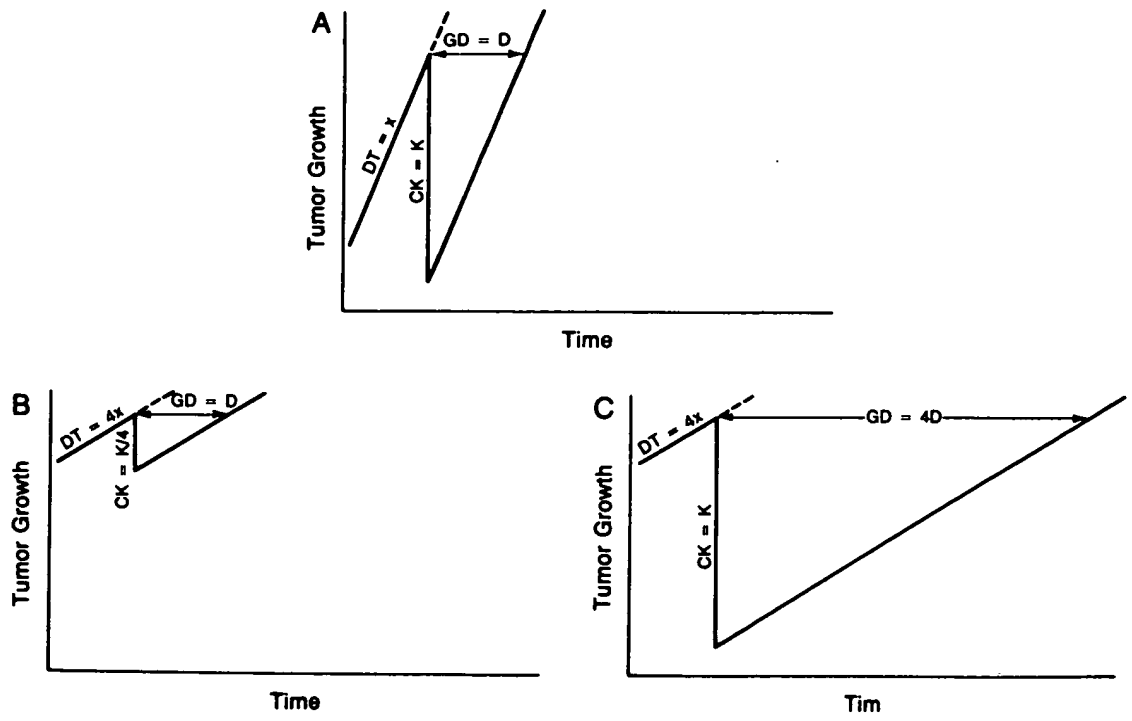


Figure 56.3. Tumor growth in r lation to time before and after treatment (**A**). A 4-fold doubling time r quires 1/4 of cell kill for the same growth delay (**B**). The same amount of cell kill results in 4-fold incr as in growth delay (**C**). DT, doubling time; CK, c ll kill; GD, growth delay; K, r lative cell kill; D, relative growth delay ov r time.

an autosomal recessive gene (50). It is responsible for the absence of hair in addition to other abnormalities including retarded growth, low fertility, and short life span (100% mortality within 25 weeks of birth and 45% mortality within 2 weeks of birth) (134). It was not until 1968 that Pantelouris noted that some of the nude mice lacked a thymus gland. These mice were found to have a homozygous mutation *nu/nu*, while both the phenotypically normal +/+ and the heterozygous *nu/+* had a thymus (124). Immunologically, the *nu/nu* athymic mice have a small number of T cells that are residual after transplacental passage from heterozygous mothers. However, these T cells do not affect the rejection of tissue transplants (or other markers of T cell function) (129). These animals preserve B cell function (147) and exhibit a higher activity of natural killer cells (73, 80). These characteristics led to widespread use of nude mice in tissue transplantation and other areas of biomedical research (50, 53, 82, 118, 135, 137), including their use in human tumor transplantation.

The success of human tumor xenografting into the nude mice and the ability to maintain the histologic and biologic identity of tumors through successive passages in vivo revolutionized many aspects of cancer research, including drug development (83, 114, 128, 140). Transplantation of tumor cell lines into nude mice can be accomplished via multiple routes: subcutaneous, intraperitoneal (59), intravenous, intracranial (58), intrasplenic, renal subcapsular, or through a new orthotopic model by site-specific organ inoculation. Each site has specific advantages and limitations.

Subcutaneous implantation is the predominant site for transplantation of human tumor into the nude mouse because of its simplicity and easy access to tumor. Indeed, it provides the mainstay for in vivo testing of the drug discovery and screening program of the NCI (120).

A tumor cell suspension is usually injected into the flank of the animal. Depending on the clonogenic potential of the tumor, between 106 and 107 cells are required for successful engraftment. Tumors usually require between a few days to a few months to grow depending on the growth rate of the cell line used. Many human tumor xenografts have been established to date, including those from most of the solid tumors affecting adults. Human colon cancer and melanoma have been passaged for the longest time in vivo. Brain tumors have proven the most difficult to maintain (40, 47). Approximately one-half of the brain tumor cell lines have been successfully xenografted into athymic mice (40).

Of interest, subcutaneous xenografts metastasize infrequently and seldom invade adjacent tissues. This may be because of the retention of some host defenses, especially natural killer cell activity (73, 80). Thus, animal survival is not a feasible endpoint for assessing drug efficacy in nude mice, since large tumor burdens prior to death may be associated with discomfort. Instead, the growth delay or the clonogenic assay would be more appropriate in this model. However, it is possible to select primary tumors or perturb the host defense mechanisms to develop models that are locally invasive or metastatic. Metastasis can be enhanced with the depletion of NK cells by pretreating the mice with cyclophosphamide, beta estradiol, or other agents (44, 48, 94).

Human tumor cells undergo kinetic changes after transplantation and passage in the nude mic . Most frequent the transplanted tumor adapted to growth in animals has shorter doubling time than the original tumor isolated from patient (149). Growth rates increase further during subsequent passages (35, 150). The vascularity of the primary and transplanted tumor also differ with transplanted tumors showing better blood supply and less necrosis. This difference could be due to selection of the most rapidly growing cells from a heterogeneous primary animal, secretion of paracrine growth factors, which induce neovascularization or simply tumor size.

Despite these changes in kinetics of invasive potential, the majority of the xenografted human tumors maintain the morphologic and biochemical characteristics of their original tumors. Therefore, it is expected that chemosensitivity would be similar in both the original and the xenografted human tumor, and that this correlation would predict for both active single agents and active drug combinations. In fact, excellent correlations can be made between average growth delay for human tumors in nude mice treated with the best available drug combinations and complete clinical response rates (46, 61). In increasing order of responsiveness, these correlations have been shown for human xenografts of non-small cell lung cancer (114, 142), colon cancer (119), breast cancer (60), small cell lung cancer (30), and malignant melanoma (51).

Renal Subcapsular Assay (RSC). Unlike the subcutaneous xenograft assay, the renal subcapsular assay has a relatively short and constant period between tumor inoculation and the appearance of a grossly palpable mass. Tumors can usually be assessed in a period of 6 days (3). Therefore, this model is particularly appropriate when a short term in vivo assay is required. Cells are inoculated as a tumor fragment, usually 1 mm in size, under the kidney capsule of the nude mouse, as first described by Bogden and colleagues in 1978 (15). These tumors maintain true morphologic, functional, and growth characteristics of the original tumor from which they were derived (2). For example, they preserve cell-cell contact, maintain the spatial relationship of the tumor, and form a more representative model of human metastasis than the subcutaneous xenograft. Therefore, tumor response can be subsequently assessed by measuring tumor size (growth assay), colony formation by surviving cells (the clonogenic assay), or simply animal survival (1, 14, 39, 42, 156).

While appealing in many ways, the renal subcapsular assay has limitations. The subcapsular area of the kidney is not a totally immunoprivileged site. When sectioned and examined microscopically, variable amounts of tumor mass represent invading lymphocytes (49, 98, 160). Thus, the immunogenicity of a given tumor in a given animal model is an important variable to control, and considerable controversy surrounds the use of this assay (1). However, as will be discussed later, it might be an ideal orthotopic model for renal cell carcinoma (see below) (111).

Intraperitoneal, Micro ncapsulated Tum r Assay

Because of the limitations of the renal subcapsular assay and its specific poor adaptability to slow-growing tumors [2],

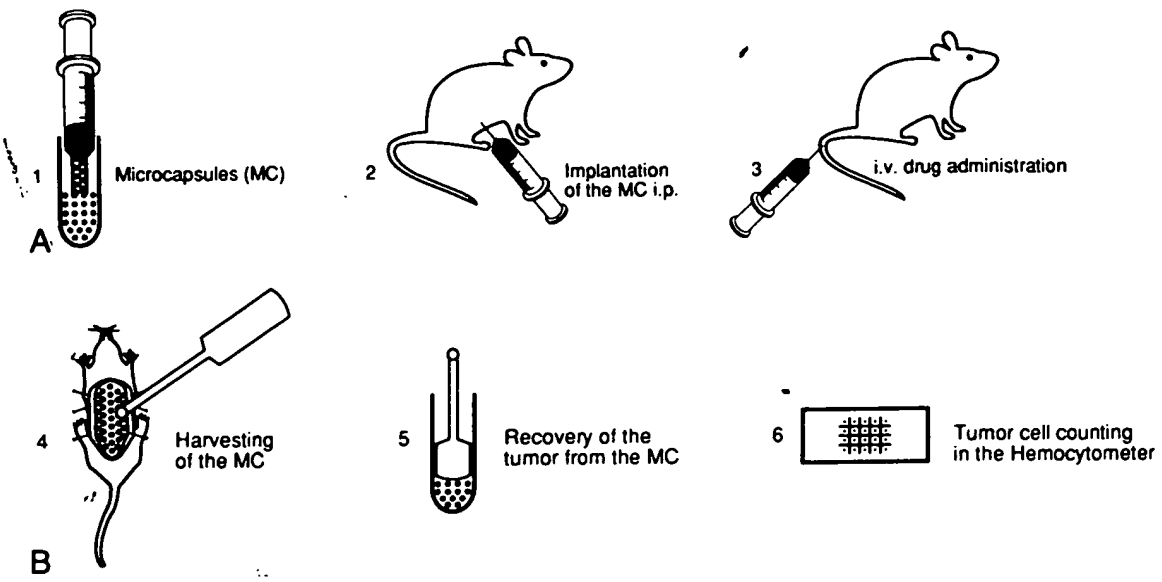


Figure 56.4. Intraperitoneal microencapsulated tumor assay.

Table 56.2. Orthotopic Models for Study of Human Cancers Grown in Athymic Nude Mice

Human cancer organ site of origin	Implantation site in nude mice	Nomenclature
Central nervous system	Percutaneous intracranial implantation into cerebral cortex	Intracranial model
Colon	Wall of cecum	Intracolonic model
Lung	Intrabronchially into right mainstem bronchus	Intrapulmonary model
	Percutaneously into right pleural space	Percutaneous intrathoracic model
Pancreas	Pancreas parenchyma	Intrapancreatic model
Renal	Subrenal capsule	Subrenal capsule model
	Kidney parenchyma	Intrarenal model

12, 43), alternative short-term in vivo assays have been developed. One of the more interesting is the microencapsulated tumor assay which depends on microencapsulation technology. Tumor cells are encapsulated in semipermeable gels that can be formed into microcapsules of (0.05 to 1 mm) (90). These microcapsules can be inoculated into the peritoneal space of experimental animals. Under typical assay conditions using mice, approximately 600 microcapsules are injected into the peritoneum. The semi-permeability of the capsule protects the tumor cells from host cell-mediated immune cytotoxicity, so that athymic (nude) mice need not be used. At the same time, it allows nutrients and systemic cytotoxic agents to diffuse and reach the tumor cells. Anticancer effect is assessed by recovering microcapsules and counting viable tumor cells in treated versus control animals (Fig. 56.4) (67, 68).

The microencapsulation assay is simple, rapid, and relatively inexpensive. For a given analysis, it requires fewer mice when compared to the subcutaneous transplanted tumor assay (68). By definition, tumor cells are evaluated after exposure to drug concentrations that are obtainable in vivo. In addition, the system is adaptable to most solid tumors and, unlike the subcutaneous transplanted tumor assay, uses immunocompetent mice. For these reasons, the microencapsulated tumor assay is being evaluated by the NCI screening program as an in vivo second line screen to follow initial drug leads that pass the in vitro screening system previously described (20).

Orthotopic Xenograft Model. In 1889, after analyzing autopsies from patients with metastatic breast cancer, Paget concluded that metastasis is not a random phenomenon. Rather, he concluded the malignant cells have special affinity for growth in the environment of certain organs, the familiar seed and soil hypothesis (123). Certainly, there exist organ site-specific interactions that are essential for optimal growth and progression of cancer in vivo (77, 106, 117, 126, 127). The orthotopic xenograft model is a system in which tumor cells are implanted at the site of the organ of origin. This organ-specific site presumably provides the tumor cells with an optimal environment for growth and progression. Because of its relevant expense and novelty, this model has as yet not been used widely by the NCI drug screening program. However, it is being used extensively to explore its role as an in vivo evaluation model for cytotoxic agents specific for organ sites such as lung cancer.

Multiple tumor xenografts have already been developed using nude mice, including renal cell carcinoma (44, 45, 112), pancreatic carcinoma (136), certain brain tumors

(139), prostate, colon, and to a larger extent lung cancer (Table 56.2) (102). All of these models are potentially amenable to orthotopic development.

The lung tumor model is the predominant orthotopic model that has been explored by the NCI102 (105), and application of other models is currently underway. In the case of lung cancer, tumor cells in suspension are inoculated through the right main stem bronchus into the right lung in a lightly anesthetized animal (Fig. 56.5). Tumor response can be evaluated by sacrificing the animal and histologically quantifying tumor growth, or as shown in Figure 56.6, non-invasive chest x-ray may be sufficient to provide interim evaluation of tumor response (104).

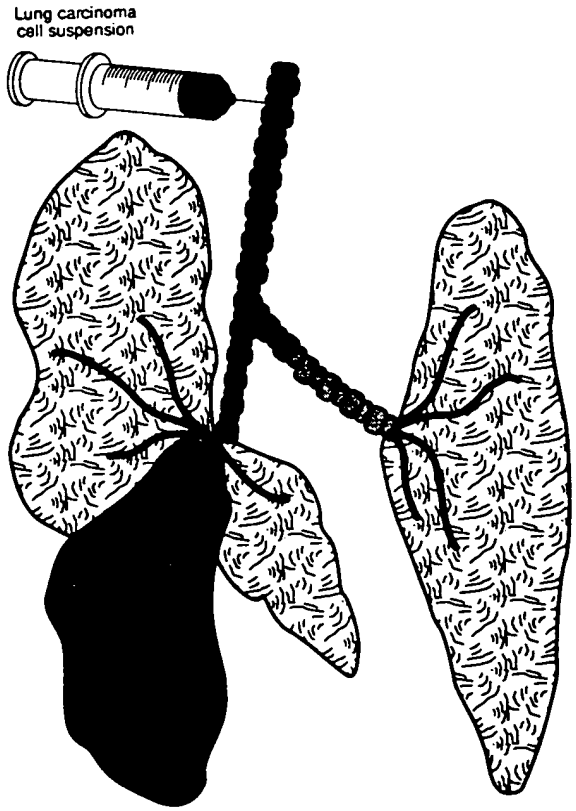


Figure 56.5. Orthotopic in vivo human lung cancer model in athymic nude mice. Intrabronchial tumor cell inoculations. *Shaded area*, tumor.

Another approach toward establishing a lung tumor orthotopic model is through percutaneous intrathoracic implantation (Fig. 56.7) (102). A disadvantage to this model is the finding that as many as 30% of the inoculated tumor grows outside the lung parenchyma, either in the pleural space or the chest wall. Tumor related mortality from the intrabronchial model is higher than that of intrathoracic implantation. Both orthotopic approaches have a much higher tumor mortality than the subcutaneous model of the same tumor cell line (102). The far greater aggressiveness of identical inoculates of lung cancer injected into the bronchus compared with subcutaneous injection is a reflection of Paget's early observation on tumor cell tissue tropism and suggests that orthotopic models may reflect the clinical situation most closely (69, 95, 110, 116).

Hollow Fiber Technology. Recently, the NCI has incorporated semipermeable hollow fiber assays into the in vivo phase of drug development. These hollow fibers allow tumor cells to grow in contact with each other, either in the log or stationary phases of cell growth (Fig. 56.8). The permeability of the fibers can be selected to limit the molecular weight of drug, which can penetrate into the tumor mass. A practical advantage of this system is that more than one tumor type can be implanted into a single animal, allowing more information to be obtained from a single in vivo experiment. In addition, the system may be adaptable to epithelial cells in an attempt to screen for compounds that interfere with angioneogenesis. Because this is an in vivo system, the agent

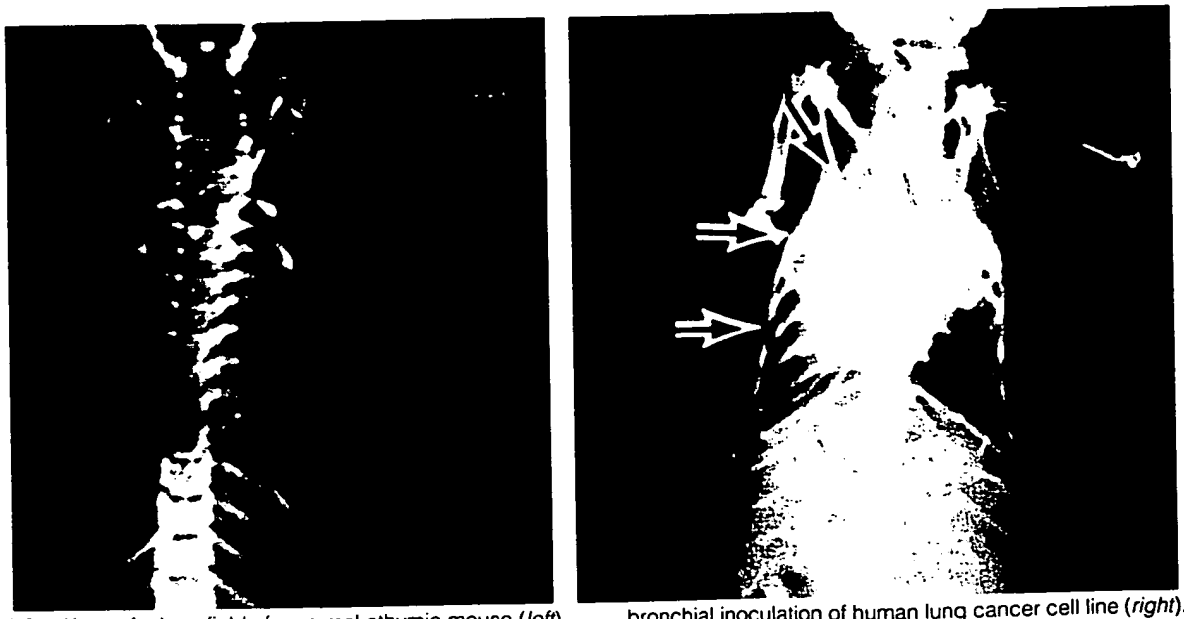


Figure 56.6. X-ray of a lung field of a normal athymic mouse (*left*) and an x-ray showing a right lung carcinoma resulted from intrabronchial inoculation of human lung cancer cell line (*right*). *Arrows*, tumor site.

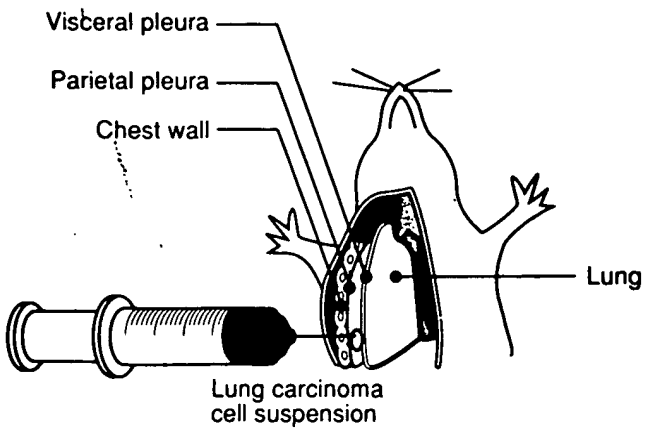


Figure 56.7. Orthotopic in vivo human lung cancer model in athymic nude mice. Percutaneous tumor cell inoculation.

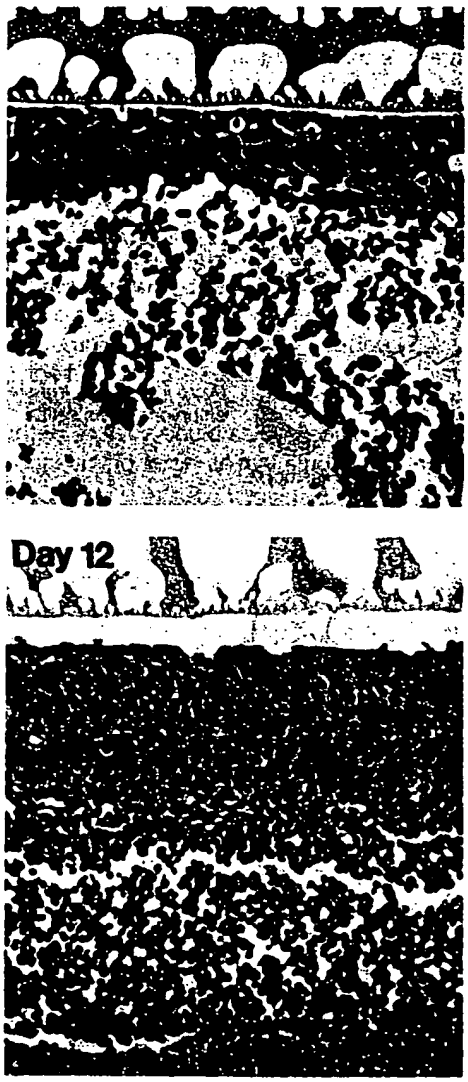


Figure 56.8. Human colon cancer cells grown in vivo in hollow fibers. Day 8 log phase growth. Day 12 stationary growth at confluence. Note central necrotic cells in the confluent tumor.

must be bioavailable to show activity, correcting for the variables of serum protein binding and drug metabolism.

Another potential advantage of hollow fiber technology is that it allows the successful maintenance of allogeneic and xenogeneic cells in immunocompetent hosts, thereby decreasing the costs associated with in vivo drug development considerably.

As currently used in the NCI program, malignant cells derived from patients with breast, kidney, lung, ovary, colon, CNS, hematologic, and melanoma cancers are encapsulated in polyvinylidene fluoride hollow fibers, which exclude molecules with a molecular weight of 500,000 or greater (80A). The fibers are then implanted either subcutaneously or intraperitoneally, with each animal hosting six samples representing three tumor cell lines each, cultured both in the peritoneal and subcutaneous spaces. Already, this technique has shown promise with known active drugs, and hollow fiber technology is increasingly being adapted to the in vivo phase of cancer drug development.

Limitations of Animal Models

IMMUNOGENICITY

The development of immunogenicity to a transplantable tumor model can complicate interpretation of treatment results. The cell kill and animal survival can become exaggerated as a result of this potential for genetic drift over time. Therefore, periodic monitoring is important to quality assurance in maintaining a stable animal model with consistent predictability.

INFECTION

Several viral infections are difficult to control in laboratory animals and require constant vigilance. These infections not only cause a decrease in the reproductive capacity but also can limit the tolerance of the animal to both tumor inoculation and therapeutic interventions. Many effects of viral infection (wasting, cachexia, or growth retardation) can mimic the dose-limiting toxicities of anticancer drugs. The most common viruses that affect laboratory mice are the mouse hepatitis virus (MHV), the Sendai virus, and the pneumonia virus of the mouse (PVM).

MHV is a major cause of death among nude mice (41). Infection can be fatal and usually (55, 56) produces cachexia and necrotizing hepatitis. Infected mice may not tolerate drugs that require hepatic clearance or that are hepatotoxic in themselves.

The Sendai virus is a common murine respiratory virus. It causes a wasting syndrome and death in immunocompromised mice (13, 41, 166). It also causes pulmonary vein thrombosis, suppurative rhinitis, and otitis media. In addition, this virus can lead to squamous metaplasia of the lung that might cause confusion in assessing tumors in these animals (130). Subclinical infection of breeding colonies can occur with no apparent symptoms (56), and continuous monitoring of animals is essential. Like the Sendai virus, PVM can induce squamous cell changes in the bronchus similar to squamous cell cancer (115). Another virus that can affect

athymic mice is th mouse leukemia virus that can caus erythroleukemia. Reovirus, polyomavirus, and ectomelia are other pathogens that can affect lab animals.

Because of the high susceptibility of the nude mice for infection, strict isolation and exclusion of infected animals from experiments is essential. In addition, microbiologic monitoring is important to maintain any reproducible experimental animal model system. Microbiologic monitoring includes routine viral isolation and serologic studies on the breeding colonies.

Other Animal Models

TRANSGENIC AND CHIMERIC MICE

The transgenic mouse is the resultant progeny of the pronucleus of a fertilized egg that is injected with a foreign gene. This progeny then carries and expresses this exogenous gene and passes it on in a Mendelian fashion to its descendants (25). Genes can be transferred to the pronucleus by microinjection (24, 66, 79, 85), retroviral infection (86–8 146), or embryonal stem cell (ESC) transfer (Fig. 56.9) (3 81, 96). By far, the most efficient of these three strategies is microinjection. ESC provides a means to manipulate and se lect cells containing the transferred gene in culture prior to insertion into animals. This is accomplished by transferring the gene into ES cells, which are then transplanted into the blastocyst to create a chimeric mouse. If reproductive tissues derived from the embryonal stem cell contribute to the germ line, a transgenic mouse is established from the progeny of the chimeric animals (Fig. 56.10) (65).

The ability to integrate a gene of interest into the genome of an animal, which then expresses it, provides a novel approach for cancer investigation. Tumorigenesis can be studied through a better understanding of interactions between regulation of expressed cellular and viral oncogenes (21). Transgenic mice are excellent models for studying the consequences of oncogene expression in animals, the effect of oncogenes on growth and differentiation, and their potential for cellular transformation. These mice also provide an in vivo preclinical model for gene therapy and gene transfer.

An example of how this technique can be applied to drug development is the recent introduction of drug resistance

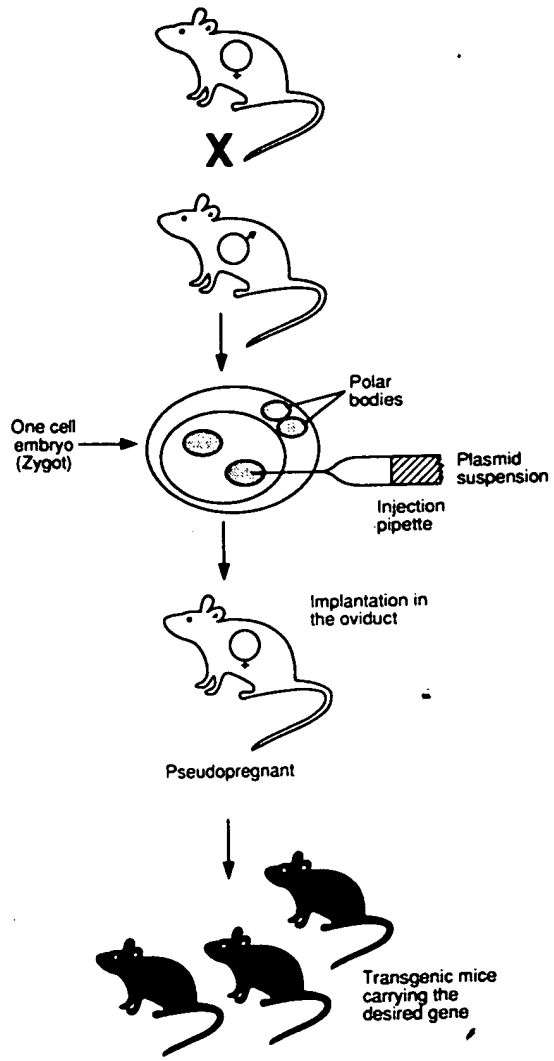


Figure 56.9. Transgenic mice production by pronuclear microinjection.

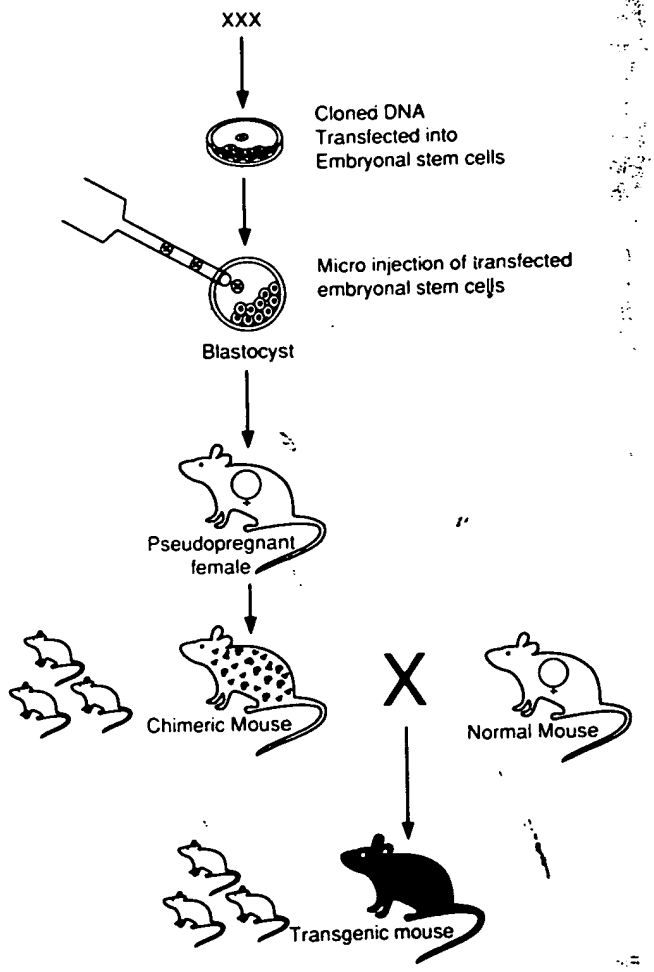


Figure 56.10. Chimeric transgenic mice derived from transfected embryonal stem cell-mediated transfer.

athymic mice is the mouse leukemia virus that can caus erythroleukemia. Reovirus, polyomavirus, and ectomelia are other pathogens that can affect lab animals.

Because of the high susceptibility of the nude mice for infection, strict isolation and exclusion of infected animals from experiments is essential. In addition, microbiologic monitoring is important to maintain any reproducible experimental animal model system. Microbiologic monitoring includes routine viral isolation and serologic studies on the breeding colonies.

Other Animal Models

TRANSGENIC AND CHIMERIC MICE

The transgenic mouse is the resultant progeny of the pronucleus of a fertilized egg that is injected with a foreign gene. This progeny then carries and expresses this exogenous gene and passes it on in a Mendelian fashion to its descendants (25). Genes can be transferred to the pronucleus by microinjection (24, 66, 79, 85), retroviral infection (86–89, 146), or embryonal stem cell (ESC) transfer (Fig. 56.9) (36, 81, 96). By far, the most efficient of these three strategies is microinjection. ESC provides a means to manipulate and select cells containing the transferred gene in culture prior to insertion into animals. This is accomplished by transferring the gene into ES cells, which are then transplanted into the blastocyst to create a chimeric mouse. If reproductive tissues derived from the embryonal stem cell contribute to the germ line, a transgenic mouse is established from the progeny of the chimeric animals (Fig. 56.10) (65).

The ability to integrate a gene of interest into the genome of an animal, which then expresses it, provides a novel approach for cancer investigation. Tumorigenesis can be studied through a better understanding of interactions between regulation of expressed cellular and viral oncogenes (21). Transgenic mice are excellent models for studying the consequences of oncogene expression in animals, the effect of oncogenes on growth and differentiation, and their potential for cellular transformation. These mice also provide an in vivo preclinical model for gene therapy and gene transfer.

An example of how this technique can be applied to drug development is the recent introduction of drug resistance

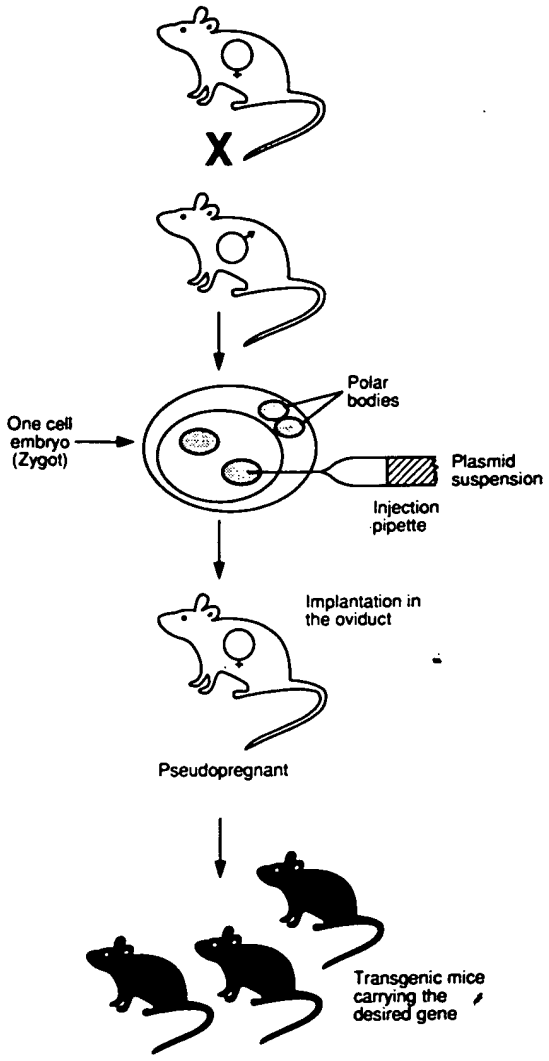
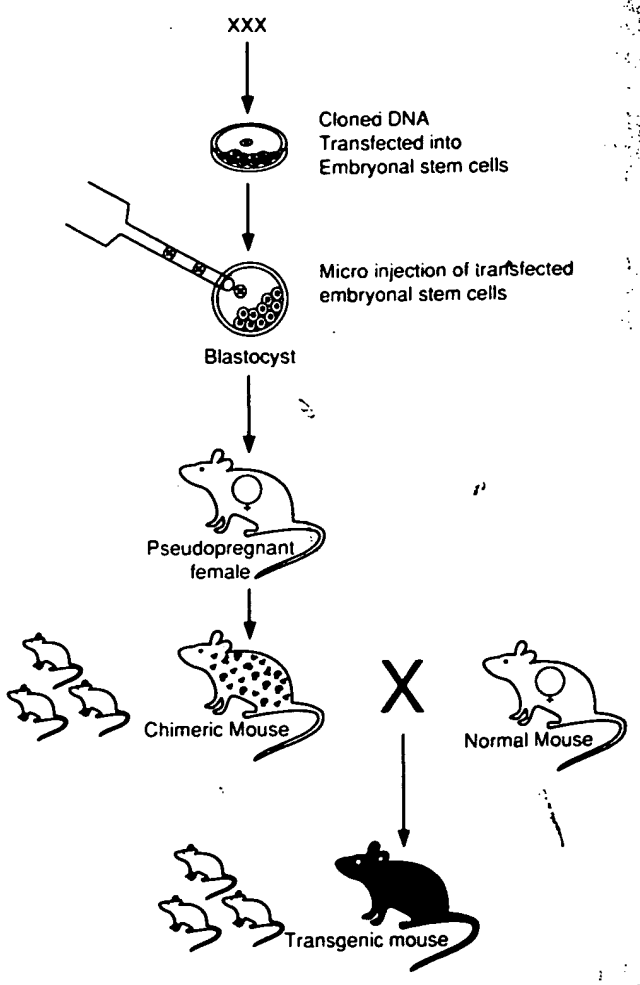


Figure 56.9. Transgenic mice production by pronuclear microinjection.



Figur 56.10. Chimeric transgenic mice derived from transfected embryonal stem cell-mediated transfer.

genes into transgenic animals. These genes include the multiple drug resistance (or *mdr* gene), which confirms resistance to a variety of important drugs of the natural product class including VP-16, Adriamycin, and the vinca alkaloids (20).

Because normal cells from transgenic mice transfected with the *mdr* gene express the same surface glycoprotein that confers drug resistance to tumor cells, they are able to tolerate normally lethal doses of anticancer drugs of the natural product class with toxicity. Such animal models may have unique roles in cancer drug development (57, 109). For example, they could be used, in an in vivo system, to screen or further evaluate drugs capable of reversing the resistance phenotype.

ANIMAL MODELS IN CANCER DRUG DEVELOPMENT

The previous section of this chapter has reviewed the role of animal models in cancer drug discovery. Following identification of a compound of interest, animal models continue to be important to the process of cancer drug development, specifically in the area of preclinical toxicology. These studies are done with a 2-fold purpose: 1) to estimate a safe starting dose for phase I clinical trials in man and 2) to predict acute and chronic toxicities in a relevant preclinical animal model. The role of the animal model has evolved in this area as well.

In the 1970s, the NCI used only dogs and monkeys in its preclinical toxicology protocols. Lethal and nonlethal doses were established in both models and chronic toxicity studies undertaken only in dogs. Starting doses for patient studies were calculated as one-third of the lowest toxic dose for the most sensitive animal model, monkey or dog (70).

In 1979, the NCI and the Food and Drug Administration reviewed existing data and agreed that toxicity studies performed largely in mice could safely replace the more costly and time-consuming large animal studies in dog and monkey models.

Currently, the LD10 (the dose of drug lethal to 10% of animals) in mice is tested in a dog model using an MELD10 or mouse equivalent LD10. This dose can be estimated from a conversion equation:

$$\text{dose (in mg/m}^2\text{) in dogs} = \frac{K_m \text{ dog}}{K_m \text{ mouse}} \times \text{dose (in mg/m}^2\text{) in mouse}$$

where K_m is the surface area to weight ratio in each species (54). This is an important equation for dose conversion between species for cancer drug development (Table 56.3). In the absence of severe toxicity in dogs, phase I trials in humans may begin at one-tenth the LD10 in mice. Of course, if severe toxicity is observed in dogs at the mouse LD10, doses are de-escalated to determine the minimally toxic dose in dogs. Clinical studies may then begin at one-third of this dose derived in dogs. Overall, the new NCI toxicology protocol has performed well in predicting safe initial doses for clinical trials, while reducing the reliance on and cost of preclinical large animal toxicology.

A new use of animals in preclinical drug development beyond simple prediction of toxicity has recently gained momentum. This is the use of preclinical pharmacology to guide dose escalation during the conduct of phase I clinical trials. As Collins and co-workers note in a recent excellent review of this concept (27), the rationale for pharmacologically guided dose escalation derives from the simple assumption that similar toxicities will occur at similar drug levels in mice and man. Since both toxicity and efficacy of anticancer drugs is related to total drug exposure, the area under the pharmacokinetic curve (AUC) has been proposed for this purpose.

Table 56.3. Surface Area to Weight Ratios (K_m) of Various Species

Species	Body weight	Surface area	Surface area to weight ratio
	(*kg*)		(K_m)
Mouse	0.02	0.0066	3.0
Rat	0.15	0.025	5.9
Monkey	3	0.24	12
Dog	8	0.40	20
Human			
Child	20	0.80	25
Adult	60	1.6	37

In essence, the AUC is measured in mice following treatment with a given drug at the LD10 dose. This is compared with the AUC in patients entering the first dose of the phase I study, which, as previously discussed, is usually one-tenth the mouse LD10. If the AUC in man is significantly lower than that observed at the LD10 in mice, dose escalation can be accelerated beyond the standard Fibonacci schema. The speed with which dose can be escalated depends upon the therapeutic index of a given agent, but two escalation schemas have been proposed. The first, a geometric mean approach, uses a dose escalation factor equal to the square root of the ratio of the AUC at the mouse LD10 to the AUC in man at the entry dose level. The second schema continues to double doses at each escalation until the AUC in man approaches that seen in the mouse at the LD10. Drug levels would continue to be monitored in all patients on study to be certain that non-linear kinetics would not cause unexpected toxicities.

This hypothesis, of course, assumes that drug metabolism and end organ sensitivity to both parent drug and metabolites are similar in mouse and man. As Collins and others have convincingly demonstrated, these assumptions generally are true so that this approach could potentially save significant time in clinical drug development. In fact, pharmacologically directed dose escalation has been successfully used to accelerate dose escalation in a number of anticancer drugs in phase I clinical trials, including HMBA, merbarone, piraxantrone, and lodoxorubicin.

Conclusions

The use of animals in cancer drug discovery and development has evolved to become both more sophisticated and efficient over the past four decades. Despite contempo-

rary interests and pressure to decrease animal use in research, it is likely that animal models will play an increasingly important role in both cancer drug discovery and development.

To be sure, it is likely that the broad-based in vivo mouse screen using sensitive murine tumor cell lines, and which required several million mice during each year of operation, has been supplanted by more targeted screening systems that no longer require an in vivo model. The current NCI human tumor cell line screen has the theoretic advantage of being able to identify compounds specifically active in a given tumor type (e.g., breast, colon, lung) or histology (e.g. adenocarcinoma or squamous cell cancer). In addition, the assay conditions of the screen will hopefully allow the identification and characterization of new natural products from novel sources.

There are other screening models that require neither animals nor living cells. These screens select biochemical targets that can be purified and then inhibited as part of a screen. Examples include the P170 glycoprotein (screening for compounds that displace active drugs from the binding site, and that could reverse the multiple drug resistance phenotype), inhibitors of DNA topoisomerases or drugs that bind to specific growth factor receptors.

While these new screening systems are now possible because of a better understanding of the biology and growth requirements of cancer cells, they do not supplant animals entirely. Once a screen of any kind has identified an interesting lead, intermediate steps requiring animals will still be required prior to clinical trials in man.

These studies at a minimum include confirming activity against a given tumor in a relevant animal model, growth delay or improved survival in nude mice, inhibition of orthotopic tumor growth, or significant cell kill in the microencapsulation model. The animal model is critical in taking the screen one step closer to the clinic. It confirms that the drug and/or its metabolites reach their target and demonstrate a positive and reproducible therapeutic effect.

While this chapter has focused on cancer drug discovery and development, animal models have a special role in the development of biologic agents. Here the relevant biologic endpoints may not cross species. For example, G-CSF does not affect bone marrow function in mice, while GM-CSF treatment induces a profound leucocytosis in mice. These agents may require animal models closer to man (non-human primates) or other systems, such as the SCID (severe combined immunodeficiency) mouse model, in which the human immune system can be selectively introduced and the effects of biological agents monitored in a controlled, yet essentially human, milieu.

Just as the role of animals in cancer drug discovery has become more refined over time, so too has their role in drug development. The general convertibility of doses between species has decreased the need for larger animals (non-human primates and dogs) during preclinical toxicology. The incorporation of pharmacokinetics into preclinical toxicology has become routine and is appealing for a number of reasons. Such studies provide insights into drug metabolism as it relates to end organ toxicity and can determine whether saturable (non-linear) kinetics contribute to the therapeutic index. Perhaps most interesting is the recent successful application of pharmacologically directed dose escalation to phase I studies in man and the refinement that this approach will give to what has been a largely empiric area of clinical research.

Appropriate use of animal models is essential to the successful, efficient and safe discovery and development of new treatments for patients with cancer. The lessons learned will hopefully have a positive influence on the development of new therapies for other diseases as well.

References

1. Aamdal S, Fostad O, Kaalhus O, Phil A. Chemosensitivity profiles of human cancers assessed by the 6-day SRC assay on serially xenografted tumors. Int J Cancer 1986;37:579.
2. Aamdal S, Fodstad O, Phil A. Human tumor xenografts transplanted under the renal capsule of conventional mice: growth rates and host immune response. Int J Cancer 1984;34:725.
3. Aamdal S, Fodstad O, Phil A. Methodological aspects of the 6-day subrenal capsule assay for measuring response of human tumors to anticancer agents. Anticancer Res 1985;5:329.
4. Ad Hoc review committee proceedings for National Cancer Institute. In Vitro/In Vivo Disease-oriented Screening Project. NCI, National Institutes of Health. Bethesda, MD, September 23–24, 1985.
5. Ad Hoc review committee proceedings for National Cancer Institute. In Vitro/In Vivo Disease-oriented Screening Project. NCI, National Institutes of Health, Bethesda, MD, December 8–9, 1986.
6. Ad Hoc review committee proceedings for National Cancer Institute. In Vitro/In Vivo Disease-oriented Screening Project. NCI, National Institutes of Health, Bethesda, MD, December 8–9, 1989.
7. Alley MC, Hursey ML, Pacula-Cox CM, Stinson SF, McLemore TL, Boyd MR. Suitability of multicellular growth units in soft agar culture for experimental drug evaluations and morphologic examinations. Proc Am Assoc Cancer Res 1989;30:529.
8. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 1988;48:589.
9. Begg AC. Analysis of growth delay data: potential pitfalls. Br J Cancer 1980;41(suppl IV):93.
10. Begg AC. Principles and practices of the tumor growth delay assay. In Rodent Tumor Models in Experimental Cancer Therapy. Edited by RF Kallman. New York: Pergamon, 1987. p 114.
11. Bellet RE, Danna V, Mastrangelo MJ, Berd D. Evaluation of a nude mouse-human tumor panel as a predictive secondary screen for cancer chemotherapeutic agents. JNCI 1979;63:1185.
12. Bennett JA, Pilon VA, MacDowell RT. Evaluation of growth and histology of human tumor xenografts implanted under the renal capsule of immunocompetent and immunodeficient mice. Cancer Res 1985;45:4963.
13. Blandford G, Cureton RJ, Heath RB. Studies of the immune response in Sendai virus infection of mice. J Med Microbiol 1971;4:351.
14. Bogden AE, Griffin W, Reich SD, Constanza ME, Cobb WR. Predictive testing with the subrenal capsule assay. Cancer Treat Rev 1984;11:113.
15. Bogden AE, Haskell PM, LePage DJ, Kelton D, Cobb WR, Esber HJ. Growth of human tumor xenografts implanted under the renal capsule of normal immunocompetent mice. Exp Cell Biol 1979;47:218.
16. Bogden AE, Kelton DE, Cobb WR, Esber HJ. In Proceedings of the Symposium on the Use of Athymic (Nude) Mice in Cancer Res. Edited by DP Houchens and AA Ovejera. New York: Gustav Fisher, 1978, p 231.
17. Boyd MR. National Cancer Institute drug discovery and development. In Accomplishments in Oncology: Cancer Therapy: Where Do We Go From Here? vol 1, no 1. Edited by E Frei and EJ Freireich. Philadelphia: Lippincott, 1986.
18. Boyd MR. NIH new drug program. Proceedings of the IV World Conference on Lung Cancer, Toronto, Canada, August 1985;25–30. Chest 1986;89:355S.
19. Boyd MR. Status of implementation of the NCI human tumor cell line in vitro primary drug screen. Proc Am Assoc Cancer Res 1989;30:652.
20. Boyd MR. Status of the NCI preclinical antitumor drug discovery screen. In Principles and Practice of Oncology Updates. Edited by VT DeVita, S Hellman, and SA Rosenberg. Philadelphia: Lippincott, 1981.
21. Boyd MR, Shoemaker R, Alley M, et al. New NCI disease-oriented drug screening program. In Proceedings of the 5th NCI-EORTC Symposium on New Drugs Cancer Therapy. Amsterdam, 1986.
22. Boyd MR, Shoemaker RH, Cragg GM, Suffness M. New avenues of investigation of marine biologicals in the anticancer drug discovery program of the National Cancer Institute. In Pharmaceuticals and the Sea. Edited by CW Rinehart, KL Rinehart, and LS Shields. Lancaster: Technomic Publishing AG 1988.
23. Boyd MR, Shoemaker RH, McLemore TL, et al. New drug development. In Thoracic Oncology. Edited by J Roth, JC Ruckdescel, and THE Weisenburger. Philadelphia: Saunders, 1989.
24. Brinster RL, Chen HY, Trumbauer ME, Yagle MK, Palmiter RD. Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. Proc Natl Acad Sci USA 1985;82:4438.
25. Brinster RL, Palmiter RD. Introduction of genes into germ line of animals. Harvey Lect 1985;80:1.
26. Bruce WR, Meeker BE, Valeriote F. A comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered in vivo. JNCI 1966;37:233.
27. Collins JM, Grieshaber CK, Chabner BA. Pharmacologically guided Phase I clinical trials based on preclinical drug development. JNCI 1990;82:1321.
28. Corbett TH, Griswold DP Jr, Roberts BJ, Peckham JC, Schabel FM Jr. Tumor induc-

tion relationships in development of transplantable cancers of the colon in mice for chemotherapy assays, with a note on carcinogen structure. Cancer Res 1975;35: 2434.
29. Corbett TH, Roberts BJ, Leopold WR, Peckham JC, Wilhoff LJ, Griswold DP Jr, Schabel FM Jr. Induction and chemotherapeutic response of two transplantable ductal adenocarcinomas of the pancreas in C57B2/6 mice. Cancer Res 1984;44:717.
30. Corbett TH, Valeriote FA, Baker LH. Is the P388 murine tumor no longer adequate as a drug discovery model? Invest New Drugs 1987;5:3.
31. Danks MK, Yalowich JC, Bech WT. Atypical multiple drug resistance in a human leukemic cell line selected for resistance to teniposide (VM-26). Cancer Res 1987;47: 1297.
32. Davis AJS, Leuchars E, Wallis V, Koler PC. The mitotic response of thymus-derived cells to antigenic stimulus. Transplantation 1966;4:4348.
33. DeVita VT, Oliverio VT, Muggia FM, et al. The drug development and clinical trials programs of the Division of Cancer Treatment, National Cancer Institute. Cancer Clin Trials 2:195;1979.
34. DeVita VT, Schein PS. The use of drugs in combination for the therapy of cancer. N Engl J Med 1973;288:998.
35. Division of Cancer Treatment Board approves new screening program, natural products concepts. Cancer Lett 1985;11:4.
36. Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R. The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. J Embryol Exp Morphol 1985;87:27.
37. Donovick R, Alley AM, Stinson S, McLemore T, Mayo J, Shoemaker R, Fiebig H, Boyd M. Current status and future development of "disease-oriented" panels of human tumor cell lines for use in the NCI anticancer drug screen. Proc Am Assoc Cancer Res 1989;30:611.
38. Driscoll JS. The preclinical new drug research program of the National Cancer Institute. Cancer Treat Rep 1984;68:63.
39. Dumont P, VanderEsch EP, Jabri M, Lejeune F, Atassi G. Chemosensitivity of human melanoma xenografts in immunocompetent mice and its histological evaluation. Int J Cancer 1984;33:447.
40. Dykes DJ, Mayo JG, Abbott BJ, Harrison SD Jr, Laster WR Jr, Simpson-Herren L, Griswold DP Jr, Boyd MR. In vivo growth characteristics of human tumor xenografts from the NCI in vitro "disease-oriented" drug discovery program. Proc Am Assoc Cancer Res 1989;30:614.
41. Eaton GJ, Outzen HC, Custer RP, Johnson FN. Husbandry of the "nude" mouse in conventional and germ-free environments. Lab Anim Sci 1975;25:309.
42. Edelstein MB, Smink T, Ruiter DJ, Visser W, Van Putten LM. Improvements and limitations of the subrenal capsule assay for determining tumour sensitivity to cytostatic drugs. Eur J Cancer Clin Oncol 1984;20:1549.
43. Edelstein MD, Fiebig HH, Smink T, Van Putten LM, Schuchhardt C. Comparison between macroscopic and microscopic evaluation of tumor responsiveness using the subrenal capsule assay. Eur J Cancer Clin Oncol 1983;19:995.
44. Fidler IJ. Rationale and methods for the use of nude mice to study the biology and therapy of human cancer metastasis. Cancer Metastasis Rev 1986;5:29.
45. Fiebig HH, Weigeldt H, Schuchhardt C, Zeschnigk C, Lohr GW. Transplantation of human tumors under the renal capsule of nude, immunocompetent and preirradiated normal mice. In Advances in the Chemotherapy of Gastrointestinal Cancer. Edited by HD Klein and H Kohn. Erlanden: perimed Fachbuch-Verlagsgesellschaft 1984, p 27.
46. Fiebig HH, Schuchhardt C, Henss H, Fiedler L, Lohr GS. Comparison of tumor response in nude mice and in the patients. Behring Inst Mitt 1984;74:343.
47. Fiebig HH, Winterhalter B, Berger D, Wittekind C, Bender K, Selby M, Bittner C, Alley M, Boyd M. Properties of 6 human tumor xenografts in vivo from which cell lines were developed. Proc Am Assoc Cancer Res 1989;30:612.
48. Fine DL, Shoemaker R, Gazdar A, Mayo JG, Fodstad O, Boyd MR, Abbott BJ, Donovan PA. Metastatic models of human tumors in athymic mice: useful models for drug development. Cancer Detect Prev (suppl) 1987;1:291.
49. Finney DJ. Statistical Method in Biological Assay, 2nd ed. London: Charles Griffin, 1964, p 524.
50. Flanagan SP. Nude, a new hairless gene with pleiotropic effects in the mouse. Genet Res 1966;8:295.
51. Fodstad O, Aas N, Phil A. Response to chemotherapy of human, malignant melanoma xenografts in athymic, nude mice. Int J Cancer 1980;25:453.
52. Fogh J, Trempe G. In Human Cells in Vitro New Human Tumor Cell Line. Edited by J Fogh. New York: Plenum, 1975, p 115.
53. Fogh J, Giovanella BC. (eds.) The Nude Mouse in Experimental and Clinical Research. New York: Academic, 1978.
54. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. Cancer Chemother Rep 1966;50:219.
55. Fujiwara K. Spontaneous virus infections of nude mice. In The Nude Mouse in Experimental and Clinical Research. Edited by J Fogh and BC Giovalla, 1982, p 1.
56. Fujiwara K, Takenaka S, Shumiya, S. Carrier state of antibody and viruses in a mouse breeding colony persistently infected with Sendai and mouse hepatitis viruses. Lab Anim Sci 26(suppl 2, part 1):153 1976.
57. Galski H, Sullivan M, Willingham MC, Chin K-V, Gottesman MM, Pastan I, Merlino GT. Expression of a human multidrug resistance cDNA (MDR1) in the bone marrow of transgenic mice: resistance to daunomycin-induced leukopenia. Mol Cell Biol 1989;9: 4357.
58. Gazdar AF, Carney DN, Sims HL, Simmons A. Heterotransplantation of small cell carcinoma of the lung into nude mice: comparison of intracranial and subcutaneous route. Int J Cancer 1981;28:777.
59. Gazdar AF, Shoemaker R, Mayo J, Oie HK, Donovan P, Fine D. Human lung cancer xenografts and metastases in athymic (nude) mice. In Immune-deficient animals in biomedical research, 5th International Workshop. Edited by N Rygaard, N Brunner, N Graem, and M Spang-Thomsen. Basel: Karger, 1987, p 277.
60. Giovanella BC, Stehlin JS, Shepard RC. Experimental chemotherapy of human breast carcinomas heterotransplanted in nude mice. In Proceedings Second International Workshop on Nude Mice. Tokyo: University of Tokyo Press, 1977, p 475.
61. Giovanella BC, Stehlin JS, Shepard RC, Williams LJ. Correlation between response to chemotherapy of human tumors in patients and in nude mice. Cancer 1983;52:1146.
62. Goldin A, Carter SK. Screening and Evaluation of Antitumor Agents. In Cancer Medicine. Edited by JF Holland and E Frei III. Philadelphia: Lea & Febiger, 1982, p 633.
63. Goldin A, Schepartz SA, Venditti JM, et al. Historical development and current strategy of the National Cancer Institute Drug Development Program. In Methods of Cancer Res, vol 16. Edited by VT DeVita, and H Busch. New York: Academic, 1979, p 165.
64. Goldin A, Venditti JM, Macdonald JS, Muggia FM, Henney JE, DeVita VT, Jr. Current results of the screening program at the Division of Cancer Treatment, National Cancer Institute. Eur J Cancer 1981;17:129.
65. Gordon JW. Transgenic animals. Int Rev Cytol 1989;115:171.
66. Gordon JW, Ruddle FH. Gene transfer into mouse embryos: production of transgenic mice by pronuclear injection. Methods Enzymol 1983;101:411.
67. Gorelik E, Alley M, Shoemaker R. A new in vivo short-term assay for evaluation of antitumor chemotherapeutic drugs. Proc Am Assoc Cancer Res 1986;27:389.
68. Gorelik E, Ovejera A, Shoemaker R, Jarvis A, Alley M, Doff R, Mayo J, Herberman R, Boyd M. Microencapsulated tumor assay: new short-term assay for in vivo evaluation of the effects of anticancer drugs on human tumor cell lines. Cancer Res 1987;47: 5739.
69. Goustin AS, Leof EB, Shipley GD, Moses HL. Growth factors and cancer. Cancer Res 1986;46:1015.
70. Grieshaber CK, Marsoni S. Relation of preclinical toxicology to findings in early clinical trials. Cancer Treatment Reports 1986;70:65.
71. Gupta RS. Genetic, biochemical and cross-resistance studies with mutants of Chinese hamster ovary cells resistant to the anticancer drugs, VM-26 and VP16–213. Cancer Res 1983;43:1568.
72. Hart IR. "Seed and soil" revisited: Mechanisms of site-specific metastasis. Cancer Met Rev 1982;1:5.
73. Herberman RB. Natural cell-mediated cytotoxicity in nude mice. In The Nude Mouse in Experimental and Clinical Research. Edited by J Fogh and BC Herbeman. New York: Academic, 1978, p 135.
74. Hill RP. An appraisal in vivo assays of excised tumours. Br J Cancer 1980;41(suppl IV):230.
75. Hill RP. Excision assay. In Rodent Tumor Models in Experimental Cancer Therapy. Edited by RF Kallman. New York: Pergamon, 1987, p 67.
76. Hill RP. The assay of tumour colonies in the lung. In Cell Clones: A Manual of Mammalian Cell Techniques. Edited by C Potten and JH Hendry. London: Churchill Livingstone, 1985, p 208.
77. Hill RP. The TD50 assay for tumour cells. In Cell Clones: A Manual of Mammalian Cell Techniques. Edited by C Potten and JH Hendry. London: Churchill Livingstone, 1985, p 223.
78. Hirschberg E. Patterns of response of animal tumors to anticancer agents. Cancer Res 1963;23:(suppl 5, Part 2):521.
79. Hogan B, Constantini F, Lacy E. Manipulating the mouse embryo: a laboratory manual. Edited by D. Hanahan. Cold Spring Harbor Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1986.
80. Holden HT, Herberman RB, Santoni A, et al. Natural cell-mediated cytotoxicity in nude mice. In Proceedings of the Symposium on the Use of Athymic Nude Mice in Cancer Res. Edited by DP Houchens and AA Ovejera. New York: Gustav Fischer 1978:p 81.
80a. Hollingshead, MG, Alley MC, CamAlier RF, Abbott BJ, Mayo JG, Malspeis L, Grever MR. In vivo cultivation of tumor cells in hollow fibers. Life Sci 57:131–141, 1995.
81. Hooper M, Hardy K, Handyside A, Hunter S, Monk M. HPRT-deficient (Lesch-Nyhan) mouse embryos derived from germline colonization by cultured cells. Nature 1987;326:292.
82. Houchens DP, Ovejera AA. (eds.). Proceedings of the Symposium on the Use of Athymic Nude Mice in Cancer Res. New York: Gustav Fischer, 1978.
83. Houghton JA, Taylor DM. Growth characteristics of human colorectal tumours during serial passage in immune-deprived mice. Br J Cancer 1978;37:213.
84. Isaacson JH, Cottanach BM. Report. Mouse Newsletter 1962;27:31.
85. Jaenisch R. Infection of mouse blastocysts with SV40 DNA: normal development of the infected embryos and persistence of SV40-specific DNA sequences in the adult animals. Cold Spring Harb Symp Quant Biol 1975;39:375.
86. Jaenisch R. Retroviruses and embryogenesis: microinjection of Moloney leukemia virus into midgestation mouse embryos. Cell 1980;19:181.
87. Jaenisch R, Kahner D, Nobis P, Simon I, Lohler J, Harbers K, Grotkopp D. Chromosomal position and activation of retroviral genomes inserted into the germ line of mice. Cell 1981;24:519.
88. Jaenisch R, Mintz B. Simian virus 40 DNA sequences in DNA of healthy adult mice derived from preimplantation blastocysts infected with viral DNA. Proc Natl Acad Sci USA 1974;71:1250.
89. Jahner D, Jaenisch R. Integration of Moloney leukaemia virus into the germ line of mice: correlation between site of integration and virus activation. Nature 1980;287: 456.
90. Jarvis AP, Grdina TA. Production of biologicals from microencapsulated living cells. BioTechniques 1983;1:22.
91. Johnson RD, Goldin A. The clinical impact of screening and other experimental tumor studies. Cancer Treat Rev 1975;2:1.
92. Jung H, Beck HP, Brammer I, Zywietz F. Depopulation and repopulation of R1H rhabdomyosarcoma of the rat after X-irradiation. Eur J Cancer 1981;17:375.
93. Kallman RF, Silini G, Van Putten LM. Factors influencing the quantitative estimation of the in vivo survival of cells from solid tumors. JNCI 1967;39:539.
94. Kerbel RS, Frost P, Liteplo R, Carlow DA, Elliot BE. Possible epigenetic mechanism of tumor progression: induction of high-frequency heritable but phenotypically unstable changes in the tumorigenic and metastatic properties of tumor cell populations by 5-azacytidine treatment. J Cell Physiol 1984;3:(suppl)87.
95. Korman LY, Carney DN, Citron ML, Moody TW. Secretin/vasoactive intestinal peptide-stimulated secretion of bombesin/gastrin releasing peptide from human small cell carcinoma of the lung. Cancer Res 1986;46:1214.
96. Kuehn MR, Bradley A, Robertson EJ, Evans MJ. A potential animal model for Lesch-Nyhan syndrome through introduction of HPRT mutations into mice. Nature 1987;326: 295.
97. Lee SS, Giovanella BC, Stehlin JS Jr, Brunn JC. Progression of human tumors established in nude mice after continuous infusion of thymidine. Cancer Res 1979;39:2928.
98. Levi FA, Blum JP, Lemaigre G, Bourut C, Reinberg A, Mathe G. A four-day subrenal capsule assay for testing the effectiveness of anticancer drugs against human tumors. Cancer Res 1984;44:2660.

99. Marsoni S, Hoth D, Simon R, Leyland-Jones B, De Rosa M, Wittes RE. Clinical Drug Development: an analysis of phase II Trials 1970–1985. Cancer Treat Rep 1987;71: 71.
100. Martin DS, Fugmann RA, Stolfi RL, Hayworth PE. Solid tumor animal model therapeutically predictive for human breast cancer. Cancer Chemother Rep 1975;5:(part 2)89.
101. McCredie JA, Inch WR, Sutherl RM. Differences in growth and morphology between the spontaneous C3H mammary carcinoma in the mouse and its syngeneic transplants. Cancer 1971;27:635.
102. McLemore TL, Abbott BJ, Mayo JG, Boyd MR. Development and application of new orthotopic in vivo models for use in the U.S. National Cancer Institute's drug screening program. In 6th International Workshop on Immunodeficient Animals in Biomedical Research. Edited by B Wu and JS Zheng. Basel: S. Karger, AG, 1988.
103. McLemore T, Alley M, Liu M, Hubbard W, Adelberg S, Czerwinski M, Yu S, Stinson S, Storeng R, Eggleston J, Boyd M. Histopathologic, biochemical and molecular genetic characterization of four newly established human pulmonary carcinoma cell lines. Proc Am Assoc Cancer Res 1989;30:225.
104. McLemore TL, Eggleston JC, Shoemaker RH, Abbott BJ, Bohlman ME, Liu MC, Fine DL, Mayo JG, Boyd MR. Comparison of intrapulmonary, percutaneous intrathoracic and subcutaneous models for the propagation of human pulmonary and nonpulmonary cancer cell lines in athymic nude mice. Cancer Res 1988;48:2880.
105. McLemore TL, Liu MC, Blacker PC, Gregg M, Alley MC, Abbott BJ, Shoemaker RH, Bohlman ME, Litterst CC, Hubbard WC. Novel intrapulmonary model for orthotopic propagation of human lung cancers in athymic nude mice. Cancer Res 1987;47: 5132.
106. McLemore TL, Liu MC, Blacker PC. Comparison of intrapulmonary, percutaneous intrathoracic and subcutaneous models for the propagation of human pulmonary and non-pulmonary cancer cell lines in athymic nude mice. Cancer Res 1988;48:2880.
107. McLemore T, Storeng R, Adelberg S, Czerwinski M, Yu S, Nhamburo P, Gonzalez F, Hines R, Boyd M. Expression of different cytochrome P450 genes in human lung cancer cell lines. Proc Am Assoc Cancer Res 1989;30:11.
108. McNally NJ, DeRonde J. Radiobiological studies of tumours in situ compared with cell survival. Br J Cancer 1980;41(suppl IV):259.
109. Mickisch GH, Merlino GT, Galski H, Gottesman MM, Pastan I. Transgenic mice that express the human multidrug-resistance gene in bone marrow enable a rapid identification of agents that reverse drug resistance. Proc Natl Acad Sci USA 1991;88:547.
110. Moody TW, Pert CB, Gazdar AF, Carney DN, Minna JD. High levels of intracellular bombesin characterize human small cell lung carcinoma. Science 1986;214:1246.
111. Naito S, von Eschenhach AC, Fidler IJ. Different growth patterns and biologic behavior of human renal cell carcinoma implanted into different organs of nude mice. JNCI 1987;78:377.
112. Naito S, von Eschenbach AC, Giavazzi R, Fidler IJ. Growth and metastasis of tumor cells isolated from a human renal cell carcinoma implanted into different organs of nude mice. Cancer Res 1986;46:4109.
113. National Cancer Institute planning to switch drug development emphasis from compound to human cancer-oriented strategy. Cancer Lett 1984;10:1.
114. Neeley JE, Ballard ET, Britt AL, Workman L. Characteristics of 85 pediatric tumors heterografted into nude mice. Exp Cell Biol 1983;51:217.
115. Nettesheim P, Schreiber H, Creasia DA, Richter CB. Respiratory infections and the pathogenesis of lung cancer. Recent Results Cancer Res 1974;44:138.
116. Nicolson G. Tumor cell instability, diversification, and progression to metastatic phenotype: from oncogene to oncofetal expression. Cancer Res 1987;47:1473.
117. Nicolson GL. Organ colonization and the cell surface properties of malignant cells. Biochim Biophys Acta 1982;695:113.
118. Nomura T, Oshawa N, Tamaoki N, et al. Proceedings of the Second International Workshop on Nude Mice. Tokyo: University of Tokyo Press, 1977.
119. Nowak K, Peckham MJ, Steel GG. Variation in response of xenografts of colorectal carcinoma to chemotherapy. Br J Cancer 1978;37:576.
120. Ovejera AA. The Use of Human Tumor Xenografts in Large-Scale Drug Screening. In Rodent Tumor Models in Experimental Cancer Therapy. Edited by R F Kallman. 1987, p 218.
121. Ovejera AA, Houchens DP. Human tumor xenografts in athymic nude mice as a preclinical screen for anticancer agents. Semin Oncol 1981;8:386.
122. Ovejera AA, Johnson RK, Goldin A. Growth characteristics and chemotherapeutic response of intravenously implanted Lewis lung carcinoma. Cancer Chemother Rep 1975;5(part 2):111.
123. Paget S. The distribution of secondary growths in cancer of the breast. Lancet 1889;1:571.
124. Pantelouris EM. Absence of a thymus in a mouse mutant. Nature 1968;217:370.
125. Pattengale PK, Stewart TA, Leder A, Sinn E, Muller W, Tepler I, Schmidt E, Leder P. Animal models of human disease. Pathology and molecular biology of spontaneous neoplasms occurring in transgenic mice carrying and expressing activated cellular oncogenes. Am J Pathol 1989;135:39.
126. Poste G. Experimental systems for analysis of the malignant phenotype. Cancer Met Rev 1982;1:141.
127. Poste G, Fidler IJ. The pathogenesis of cancer metastasis. Nature 1980;283:139.
128. Povlsen CO, Rygaard J. Heterotransplantation of human adenocarcinoma of the colon and rectum to the nude mouse: a study of nine consecutive transplantations. Acta Pathol Microbiol Scand 1971;79:159.
129. Raff MC, Wortis HH. Thymus dependence of Theta-bearing cells in the peripheral lymphoid tissue of mice. Immunology 1970;18:1931.
130. Richter CB. In "Morphology of Experimental Respiratory Carcinogenesis." AEC Symposium Series 1971;21:365.
131. Rockwell S. In vivo-in vitro tumor systems: new models for studying the response of tumors to therapy. Lab Anim Sci 1977;27:831.
132. Rockwell S. In vivo-in vitro tumour cell lines: characteristics and limitations as models for human cancer. Br J Cancer 1980;41(suppl IV):118.
133. Rockwell SC, Kallman RF, Fajardo LF. Characteristics of a serially transplanted mouse mammary tumor and its tissue-culture adapted derivative. JNCI 1972;49:735.
134. Rygaard J, Povlsen CO. Athymic (nude) mice. In The Mouse in Biochemical Research, vol. VI. Edited by HL Foster, JD Small, and JG Fox. New York: Academic Press, 1982, p 51.
135. Rygaard J, Povlsen CO. (eds.). Bibliography of the Nude Mouse. Stuttgart/New York: Gustav Fischer Verlag, 1977.
136. Rygaard J, Povlsen CO. Heterotransplantation of a human malignant tumor in nude mice. Acta Pathol Microbiol Scand 1969;77:758.
137. Rygaard J, Povlsen CO. (eds.). Proceedings of the First International Workshop on Nude Mice. Stuttgart: Gustav Fischer Verlag, 1974.
138. Schepartz SA. Memorandum to suppliers of compounds. In Methods of Development of New Anticancer Drugs, National Cancer Institute Monograph 45, DHEW Publication (NIH) 76-1037, 1977, p 155.
139. Shapiro WR, Basler GA, Chernick NL, Posner JB. Human brain tumor transplantation into nude mice. JNCI 1979;62:447.
140. Sharkey FE, Fogh J, Hajdu S, Fitzgerald P, Fogh J. Experience in surgical pathology with human tumor growth in the nude mouse. In The Nude Mouse in Experimental and Clinical Research. Edited by J Fogh and B Giovanella. New York: Academic, 1978, p 188.
141. Shoemaker RH, Monks A, Alley MC, et al. Development of human tumor cell line panels for use in disease-oriented drug screening. In Prediction of Response to Cancer Chemotherapy. Edited by T Hall. New York: Alan Liss, 1988.
142. Shorthouse AJ, Peckham MJ, Smyth JF, Steel GG. The therapeutic response of bronchial carcinoma xenografts: a direct patient-xenograft comparison. Br J Cancer 1980;41(suppl IV):142.
143. Shorthouse AJ, Smyth JF, Steel GG, Ellison M, Mills J, Peckham MJ. The human tumour xenograft—a valid model in experimental chemotherapy? Br J Surg 1980;67: 715.
144. Siemann DW. Satisfactory and unsatisfactory tumor models: factors influencing the selection of a tumor model for experimental evaluation. In Rodent Tumor Models in Experimental Cancer Therapy. Edited by RF Kallman. 1987, p 12.
145. Skipper HE, Schmidt LH. Background: description of criteria, and presentation of quantitative therapeutic data on various classes of drugs obtained in diverse experimental tumor systems. Cancer Chemother Rep 1962;17:1.
146. Soriano P, Jaenisch R. Retroviruses as probes for mammalian developments: allocation of cells to the somatic and germ cell lineages. Cell 1986;46;19.
147. Sprent J, Miller J. Thoracic duct lymphocytes from nude mice: migratory properties and life span. Eur J Immunol 1972;2:384.
148. Staquet MJ, Byar DP, Green SB, Rozencweig M. Clinical predictivity of transplantable tumor systems in the selection of new drugs for solid tumors: rationale for a three-stage strategy. Cancer Treat Rep 1983;67:753.
149. Steel GG. Growth Kinetics of Tumours. Oxford: Oxford University Press, 1977.
150. Steel GG, Courtenay VD, Peckham MJ. The response to chemotherapy of a variety of human tumour xenografts. Br J Cancer 1983;47:1.
151. Steel GG, Adams K, Hodgett J, Janik P. Cell population kinetics of a spontaneous rat tumor during serial transplantation. Br J Cancer 1971;25:802.
152. Steel GG, Stephens TC. Stem cells in tumours. In Stem Cells: Their Identification and Characterization. Edited by CS Pottern. London: Churchill Livingstone, 1983, p 271.
153. Stephens TC. Measurement of tumor cell surviving faction and absolute numbers of clonogens per tumor in excision assays. In Rodent Tumor Models in Experimental Cancer Therapy. Edited by RF Kallman. New York: Pergamon Press, 1987, p 90.
154. Stephens TC, Currie GA, Peacock JH. Repopulation of irradiated Lewis lung carcinoma by malignant cells and host macrophage progenitors. Br J Cancer 1978;38. 573.
155. Stinson SF, Alley MC, Kenney S, Fiebig S, Boyd MR. Morphologic characterization of human carcinoma cell lines. Proc Am Assoc Cancer Res 1989;30:613.
156. Stratton JA, Kucera PR, Micha JP, Rettenmaier MA, Braly PS, Berman ML, Di Saia PJ The subrenal capsule tumor implant assay as predictor of clinical response to chemotherapy: 3 years of experience. Gynecol Oncol 1984;19:336.
157. Thomlinson RH. An experimental method for comparing treatments of intact malignant tumours in animals and its application to the use of oxygen in radiotherapy. Br J Cancer 1980;14:555.
158. Toolan HW. Successful subcutaneous growth and transplantation for human tumors in X-irradiated laboratory animals. Proc Soc Exp Biol Med 1951;77:572.
159. Toolan HW. Transplantable human neoplasms maintained in cortisone-treated laboratory animals: HS #1, HEP #1, HEP #2, HEP #3, and HENBRH #1. Cancer Res 1954;14:660.
160. Tueni EA, Dumont P, Jacobovitz D, Massi G, Rocmans P, Lejeune F, de Franquer P Semal P, Klastersky J. Subrenal capsule assay for fresh human tumors in immunocompetent mice: an inappropriate technique for non-small cell lung cancer. Eur J Cancer Clin Oncol 1987;23:1163.
161. Uvarov O. Research with animals: requirements, responsibilities, welfare. Lab Anim 1985;19:51.
162. Venditti JM. Foreword. In Proceedings of the Symposium on the Use of Athymic Nude Mice in Cancer Res. Edited by DP Houchens and AA Ovejera. New York: Gustav Fischer, 1978.
163. Venditti JM. Preclinical drug development: rationale and methods. Semin Oncol 1981;8:349.
164. Venditti JM. The model's dilemma. In Design of Models for Testing Cancer Chemotherapeutic Agents. Edited by IJ Fidler and RJ White. New York: Van Nostranc Reinhold, 1981, p 80.
165. Venditti JM. The National Cancer Institute Drug Discovery Program current and future perspectives. A commentary. Cancer Treat Rep 1983;67:767.
166. Ward JM, Houchens DP, Collins MJ, Young DM, Reagan RL. Naturally-occurring Sendai virus infection of athymic nude mice. Vet Pathol 1976;13:36.
167. Workshop on "Disease-oriented Antitumor Drug Discovery and Development," NIH Bethesda MD, January 9–10, 1985. Sponsored by Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.
168. Zubrod CG. Chemical control of cancer. Proc Natl Acad Sci USA 1972;69:1042.
169. Zubrod CG, Schepartz S, Leiter J, et al. The Chemotherapy Program of the National Cancer Institute: History, Analysis and Plans. Cancer Chemother Rep 1966;50:349.